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| (54) Title: SPERM ANTIGEN CORRESPONDING TO A SPERM ZONA BINDING PROTEIN AUTOANTIGENIC EPITOPE (57) Abstract The present invention involves DNA encoding a mammalian Sp 17 protein, particularly human Sp 17 protein, or antigenic peptides which are fragments thereof, along with said antigenic fragments. These proteins and peptides are useful as immunocontraceptive agents and/or for the diagnosis of autoimmune infertility. Avirulent host cells which express the antigenic proteins or peptides and which are useful as immunocontraceptive agents are also disclosed. | | |

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**SPERM ANTIGEN CORRESPONDING TO A SPERM
ZONA BINDING PROTEIN AUTOANTIGENIC EPITOPE**

This invention was made with Government Support under Grant No. U54 HD29009 from the National Institutes of Health. The Government has certain rights to this invention.

5

Field of the Invention

The present invention relates to antigens which correspond to autoantigenic epitopes on a sperm zona pellucida binding protein, along with therapeutic and diagnostic methods employing the same.

10

Background of the Invention

Autoantigens are tissue components of an organism to which that organism directs an immune response. The condition which results from such a self-directed immune response is known as autoimmunity (or
15 "autoallergy"). Proteins on or on sperm are known to be potent autoantigens, and autoimmunity to such proteins is believed a significant cause of infertility.

R. Shabanowitz and M. O'Rand, Ann. NY Acad. Sci. 541, 621-632 (1988), at Figure 7, describes various
20 human proteins which have affinity for human zona pellucida.

M. O'Rand and E. Widgren, U.S. Patent No. 5,175,148, discloses a sperm antigen which corresponds to an autoantigenic epitope of Rabbit Sperm Membrane
25 Autoantigen (RSA). RSA is now known to be a family of

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four low molecular weight glycoproteins (RSA-1,2,3,4: 14K, 16K, 17K, 18K) which function as high affinity zona binding proteins. The cloning of rabbit RSA 3 (also called "sp17") is described in R. Richardson and M. O'Rand, *Mol. Biol. Cell.* 3, 15a (1992).

Summary of the Invention

A first aspect of the present invention is isolated DNA encoding a human Sp 17 protein or antigenic peptides which are fragments thereof selected from the group consisting of: (a) isolated DNA (e.g., the DNA of SEQ ID NO:1) which encodes the human Sp 17 protein having the amino acid sequence given herein as SEQ ID NO:2; (b) isolated DNA which hybridizes to isolated DNA of (a) above and which encodes a human Sp 17 protein; (c) isolated DNA differing from the isolated DNAs of (a) and (b) above in nucleotide sequence due to the degeneracy of the genetic code, and which encodes a human Sp 17 protein; and (d) fragments of the foregoing which encode antigenic peptides (i.e., peptides which bind antibodies which bind to human Sp 17 protein, as described below, typically peptides of from 6 to twenty-five amino acids in length). These proteins and peptides are sometimes referred to as the "antigen" below, and are useful as immunocontraceptive agents and/or for the diagnosis of autoimmune infertility, as discussed below.

A second aspect of the present invention is a recombinant DNA sequence comprising vector DNA and a DNA as given above.

A third aspect of the present invention is a host cell containing a recombinant DNA sequence as given above and capable of expressing the encoded Sp 17 protein or antigenic peptide fragment thereof.

A fourth aspect of the present invention is antigenic peptides (these also being referred to as the "antigen" or "antigens" hereinbelow), useful as an immunocontraceptive agent or for the diagnosis of autoimmune infertility, selected from the group

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consisting of (1) antigenic fragments of mammalian Sp17 protein which are from six to twenty-five amino acids in length and (2) the antigenic equivalents thereof, said antigenic equivalents being: (a) a modified fragment comprising said antigenic fragment modified by the replacing of one or more amino acids to the sequence thereof; or (b) a longer peptide which incorporates the sequence of said antigenic fragment and which has (i) up to four extra amino acid residues attached to the C-terminal end of said sequence, (ii) up to four extra amino acid residues attached to the N-terminal end of said sequence, or (iii) up to four extra amino acid residues attached to the C-terminal end of said sequence and up to four extra amino acid residues attached to the N-terminal end of said sequence; wherein said antigenic equivalents bind to antibodies which to said antigenic fragments of mammalian Sp17 protein. The antigenic fragments of mammalian Sp17 protein are selected from the group consisting of (i) amino acid 4 to amino acid 28 fragments, (ii) amino acid 34 to amino acid 49 fragments, (iii) amino acid 55 to amino acid 82 fragments, (iv) amino acid 117 to amino acid 137 fragments, and (v) fragments of (i) through (iv) which are at least six amino acids in length.

25 A fifth aspect of the present invention is an immunocontraceptive method which comprises administering to a subject (e.g., a female subject) an antigen as described above in an amount effective to reduce the fertility of the subject.

30 A sixth aspect of the present invention is an immunocontraceptive vaccine formulation comprising an antigen as given above, in an amount effective to reduce the fertility of a subject, in combination with a pharmaceutically acceptable carrier.

35 A seventh aspect of the present invention is a method of screening for autoimmune infertility in a subject (e.g., a male subject), comprising detecting the

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presence of antibodies in the subject which bind to an antigen as given above, the presence of said antibodies indicating the subjected is afflicted with autoimmune infertility.

5 An eighth aspect of the present invention is an avirulent host cell (e.g., a microbial host cell) containing a recombinant DNA sequence encoding an antigen as given above and capable of expressing the encoded antigen.

10 A ninth aspect of the present invention is an immunocontraceptive vaccine formulation comprising an avirulent host cell as described above, in an amount effective to reduce the fertility of a subject, in combination with a pharmaceutically acceptable carrier.

15 In a preferred embodiment of the foregoing, the avirulent microbe lacks a functioning native chromosomal gene encoding β -aspartate semialdehyde dehydrogenase (asd), and further comprises a recombinant gene encoding a functional asd polypeptide. The recombinant gene is
20 linked to one or more genes encoding one or more antigen as given above. The avirulent microbe may also include a mutated cya gene such that the microbe is substantially incapable of producing functional adenylate cyclase, and/or a mutated crp gene, rendering the microbe
25 substantially incapable of producing functional cyclic AMP receptor protein.

The present invention is explained in greater detail in the drawings herein and the specification set forth hereinbelow.

30 Brief Description of the Drawings

Figure 1 shows the binding of antiserum from a male rabbit injected with his own sperm on binding to the rabbit Sp17 sequential decapeptides having the amino acid sequences disclosed herein as SEQ ID NO:3 through SEQ ID
35 NO:49. In this figure, the SEQ ID NO for each decapeptide is given to the left and/or right thereof.

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Figure 2 shows the binding of a pool of sera from four vasectomized men with high titers of antisperm antibodies tested against the rabbit Sp17 sequential decapeptides used in Fig 1. All the peaks on this graph
5 represent human autoantigenic, B-cell epitopes.

Figure 3 shows the binding of immune sera from a femal rabbit immunized with rabbit Sp17 recombinant antigen to the sequential decapeptides described in Fig. 1.

10 Figure 4 shows the binding of immune sera taken from a male rabbit immunized with the synthetic peptide G22C to the sequential decapeptides described in Fig. 1.

Figure 5 shows the binding of immune sera taken from a female rabbit immunized with the synthetic peptide
15 G22C to the sequential decapeptides described in Fig. 1.

Figure 6 illustrates the effect of immunization of mice with recombinant Sp17 (fusion protein) on fertility. Six mice received received human Sp17. Adjuvant controls (n=12) received TITERMAX™ adjuvant
20 (available from Sigma Co., St. Louis) only. Six mice received no injections. The mice immunized with the human Sp17 recombinant protein showed a 42% decrease in pregnancy.

Figure 7 gives the alignment of the rabbit
25 (RABSP17), mouse (MUSSP17), and human (HUMSP17) Sp17 protein sequences. Autoantigenic fragments are indicated in the boxes. Numbering is from N-terminus to C-terminus, based on the numbering of the human sequence, with gaps introduced into the other mammalian sequences
30 to maximize alignment of the autoantigenic fragments shown in the boxes, and numbers skipped where gaps are introduced so that the numbering of the autoantigenic fragments indicated in the boxes corresponds across species.

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Detailed Description of the Invention

Amino acid sequences disclosed herein are presented in the amino to carboxy direction, from left to right. The amino and carboxy groups are not presented in the sequence. Nucleotide sequences are presented herein by single strand only, in the 5' to 3' direction, from left to right. Nucleotides and amino acids are represented herein in the manner recommended by the IUPAC-IUB Biochemical Nomenclature Commission, or (for amino acids) by three letter code, in accordance with 37 CFR §1.822 and established usage. See, e.g., PatentIn User Manual, 99-102 (Nov. 1990) (U.S. Patent and Trademark Office, Office of the Assistant Commissioner for Patents, Washington, D.C. 20231); U.S. Patent No. 4,871,670 to Hudson et al. at Col. 3 lines 20-43 (applicants specifically intend that the disclosure of this and all other patent references cited herein be incorporated herein by reference).

A. Molecular Biology

DNAs which encode human Sp 17 proteins, whether they are cDNAs or genomic DNAs, encode a protein of about 17 Kilodaltons which binds to human zona pellucida at high affinity by binding sulfated, complex carbohydrates. This definition is intended to encompass natural allelic variations in the DNAs.

DNAs encoding Sp 17 proteins which hybridize to the DNA encoding the human Sp 17 protein disclosed herein, may be of any species of origin, including murine (mouse, rat), rabbit, cat, porcine, human, monkey, or baboon, but preferably code for an Sp 17 protein of mammalian origin, and most preferably code for human Sp 17 proteins. Synthetic DNAs may be made in accordance with known techniques.

Hybridization conditions which will permit other DNA sequences which code on expression for an Sp 17 protein to hybridize to a DNA sequence as given herein are, in general, high stringency conditions. For

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example, hybridization of such sequences may be carried out under conditions represented by a wash stringency of 0.3 M NaCl, 0.03 M sodium citrate, 0.1% SDS at 60°C or even 70°C to DNA disclosed herein (e.g., SEQ ID NO:1) in a standard in situ hybridization assay. (See J. Sambrook et al., Molecular Cloning, A Laboratory Manual (2d Ed. 1989) (Cold Spring Harbor Laboratory)).

In general, DNA sequences which code for Sp 17 proteins and hybridize to the DNA sequence encoding the human Sp 17 protein disclosed herein will be at least 70%, 75%, 80%, 85%, 90%, or even 95% homologous or more with the sequence of the DNA encoding the human Sp 17 protein disclosed herein.

In general, DNA sequences which encode human Sp 17 proteins which hybridize to the DNA encoding the human Sp 17 protein disclosed herein will be 93%, 94%, 95%, 96%, or even 97% homologous or more to the DNA sequence encoding the human Sp 17 protein disclosed herein.

Further, DNA sequences which code for the same Sp 17 protein as coded for by the foregoing sequences, but which differ in codon sequence from these due to the degeneracy of the genetic code, are also an aspect of this invention. The degeneracy of the genetic code, which allows different nucleic acid sequences to code for the same protein or peptide, is well known in the literature. See e.g., U.S. Patent No. 4,757,006 to Toole et al. at Col. 2, Table 1.

The production of cloned genes, recombinant DNA, vectors, transformed host cells, proteins and protein fragments by genetic engineering is well known. See, e.g., U.S. Patent No. 4,761,371 to Bell et al. at Col. 6 line 3 to Col. 9 line 65; U.S. Patent No. 4,877,729 to Clark et al. at Col. 4 line 38 to Col. 7 line 6; U.S. Patent No. 4,912,038 to Schilling at Col. 3 line 26 to Col. 14 line 12; and U.S. Patent No. 4,879,224 to Wallner at Col. 6 line 8 to Col. 8 line 59.

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A vector is a replicable DNA construct. Vectors are used herein either to amplify DNA encoding Sp 17 proteins as given herein and/or to express DNA which encodes Sp 17 proteins as given herein. An expression
5 vector is a replicable DNA construct in which a DNA sequence encoding a Sp 17 protein is operably linked to suitable control sequences capable of effecting the expression of the DNA sequence in a suitable host. The need for such control sequences will vary depending upon
10 the host selected and the transformation method chosen. Generally, control sequences include a transcriptional promoter, an optional operator sequence to control transcription, a sequence encoding suitable mRNA ribosomal binding sites, and sequences which control the
15 termination of transcription and translation. Typical vectors include, but are not limited to, plasmids, viruses, phage, and integratable DNA fragments (i.e., fragments integratable into the host genome by recombination).

20 DNA regions are operably linked or operably associated when they are functionally related to each other. For example, a promoter is operably linked to a coding sequence if it controls the transcription of the sequence; or a ribosome binding site is operably linked
25 to a coding sequence if it is positioned so as to permit translation.

Transformed host cells are cells which have been transformed or transfected with vectors containing a DNA sequence as disclosed herein constructed using
30 recombinant DNA techniques. Transformed host cells ordinarily express the receptor, but host cells transformed for purposes of cloning or amplifying the receptor DNA do not need to express the receptor.

Suitable host cells include prokaryote, yeast
35 or higher eukaryotic cells such as mammalian cells and insect cells. Cells derived from multicellular organisms are a particularly suitable host for recombinant Sp 17

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protein synthesis, and mammalian cells are particularly preferred. Propagation of such cells in cell culture has become a routine procedure (Tissue Culture, Academic Press, Kruse and Patterson, editors (1973)). Examples of
5 useful host cell lines are VERO and HeLa cells, and Chinese hamster ovary (CHO) cell lines. Expression vectors for such cells ordinarily include (if necessary) an origin of replication, a promoter located upstream from the DNA encoding the Sp 17 protein to be expressed
10 and operatively associated therewith, along with a ribosome binding site, an RNA splice site (if intron-containing genomic DNA is used), a polyadenylation site, and a transcriptional termination sequence.

The transcriptional and translational control
15 sequences in expression vectors to be used in transforming vertebrate cells are often provided by viral sources. For example, commonly used promoters are derived from polyoma, Adenovirus 2, and Simian Virus 40 (SV40). See, e.g., U.S. Patent No. 4,599,308.

20 An origin of replication may be provided either by construction of the vector to include an exogenous origin, such as may be derived from SV40 or other viral source (e.g. Polyoma, Adenovirus, VSV, or BPV), or may be provided by the host cell chromosomal replication
25 mechanism. If the vector is integrated into the host cell chromosome, the latter is often sufficient.

Rather than using vectors which contain viral origins of replication, one can transform mammalian cells by the method of cotransformation with a selectable
30 marker and the receptor DNA. Examples of suitable selectable markers are dihydrofolate reductase (DHFR) or thymidine kinase. This method is further described in U.S. Pat. No. 4,399,216.

Host cells such as insect cells (e.g., cultured
35 *Spodoptera frugiperda* cells) and expression vectors such as the baculovirus expression vector may be employed in

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carrying out the present invention, as described in U.S. Patents Nos. 4,745,051 and 4,879,236 to Smith et al.

Prokaryote host cells include gram negative or gram positive organisms, for example *Escherichia coli* (E. coli) or *Bacilli*.

Eukaryotic microbes such as yeast cultures may also be transformed with vectors carrying the isolated DNA's disclosed herein. see, e.g., U.S. Patent No. 4,745,057. *Saccharomyces cerevisiae* is the most commonly used among lower eukaryotic host microorganisms, although a number of other strains are commonly available.

B. Peptides

One group of example antigenic fragments of the present invention, illustrated by Figure 1 herein, are antigenic fragments selected from the group consisting of peptides having the amino acid sequence given herein as: SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:34, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:43, and fragments thereof which are at least six amino acids in length. Of these, particularly preferred are antigenic fragments selected from the group consisting of peptides having the amino acid sequence given herein as: SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:43, and fragments thereof which are at least six amino acids in length.

Another group of example antigenic fragments of the present invention, illustrated by Figure 2 herein, are antigenic fragments selected from the group consisting of peptides having the amino acid sequence

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given herein as: SEQ ID NO;3, SEQ ID NO;6, SEQ ID NO;7, SEQ ID NO;8, SEQ ID NO;9, SEQ ID NO;10, SEQ ID NO;11, SEQ ID NO;12, SEQ ID NO;13, SEQ ID NO;14, SEQ ID NO;15, SEQ ID NO;16, SEQ ID NO;17, SEQ ID NO;20, SEQ ID NO;21, SEQ ID NO;22, SEQ ID NO;23, SEQ ID NO;24, SEQ ID NO;25, SEQ ID NO;26, SEQ ID NO;27, SEQ ID NO;28, SEQ ID NO;29, SEQ ID NO;30, SEQ ID NO;32, SEQ ID NO;33, SEQ ID NO;34, SEQ ID NO;35, SEQ ID NO;36, SEQ ID NO;38, SEQ ID NO;39, SEQ ID NO;40, SEQ ID NO;43, SEQ ID NO;47, SEQ ID NO;48, SEQ ID NO;49, and fragments thereof which are at least six amino acids in length. Of these, particularly preferred are antigenic fragments selected from the group consisting of peptides having the amino acid sequence given herein as: SEQ ID NO;8, SEQ ID NO;9, SEQ ID NO;14, SEQ ID NO;15, SEQ ID NO;16, SEQ ID NO;21, SEQ ID NO;22, SEQ ID NO;24, SEQ ID NO;25, SEQ ID NO;34, SEQ ID NO;40, SEQ ID NO;43, and fragments thereof which are at least six amino acids in length.

In general, longer peptides preferably include the sequence of an antigenic peptide as described above. Longer peptides provide the antigenic sequence in an exposed position on the molecule, and not buried in the interior of the molecule where it would be unavailable for a binding event. Longer peptides which add not more than four additional amino acids to either the N terminal or C terminal of the antigen are preferred because sequences of such length are generally insufficient to provide an additional epitope on the longer peptide which might be detrimental to the activity of the antigen.

Peptides which may be used to carry out the present invention include analogs thereof. As used herein, analogs are those compounds which, while not having amino acid sequences identical to those of the peptides described above, have a similar three-dimensional structure. In protein molecules which interact with a receptor, the interaction between the protein and the receptor must take place at the surface-

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accessible sites in a stable three-dimensional molecule. By arranging the critical binding site residues in an appropriate conformation, peptides which mimic the essential surface features of the peptides of the present invention are designed and synthesized in accordance with known techniques. Methods for determining peptide three-dimensional structure and analogs thereto are known, and are sometimes referred to as "rational drug design techniques". See, e.g., U.S. Patent No. 4,833,092 to Geysen; U.S. Patent No. 4,859,765 to Nestor; U.S. Patent No. 4,853,871 to Pantoliano; U.S. Patent No. 4,863,857 to Blalock; (applicants specifically intend that the disclosures of all U.S. Patent references cited herein be incorporated by reference herein in their entirety). See also Waldrop, *Science*, 247, 28029 (1990); Rossmann, *Nature*, 333, 392-393 (1988); Weis et al., *Nature*, 333, 426-431 (1988). Techniques for constructing and screening libraries of peptide sequences to identify peptides that specifically bind to a given protein are known. Scott and Smith, *Science*, 249, 386-390 (1990); Devlin et al., *Science*, 249, 404-406 (1990). Further, those skilled in the art will appreciate that minor deletions or substitutions may be made to the amino acid sequences of peptides of the present invention without unduly adversely affecting the activity thereof. Thus, peptides containing such deletions or substitutions are a further aspect of the present invention.

In peptides containing substitutions or replacements of amino acids, one or more amino acids of a peptide sequence may be replaced by one or more other amino acids which does not affect the antigenicity of that sequence. Such changes can be guided by known similarities between amino acids in physical features such as charge density, hydrophobicity/hydrophilicity, size and configuration, so that amino acids are substituted with other amino acids having essentially the same functional properties. For example:

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Ala may be replaced with Val or Ser;

Val may be replaced with Ala, Leu, Met, or Ile, preferably Ala or Leu;

Leu may be replaced with Ala, Val or Ile, preferably Val or Ile;

Gly may be replaced with Pro or Cys, preferably Pro;

Pro may be replaced with Gly, Cys, Ser, or Met, preferably Gly, Cys, or Ser;

Cys may be replaced with Gly, Pro, Ser, or Met, preferably Pro or Met;

Met may be replaced with Pro or Cys, preferably Cys;

His may be replaced with Phe or Gln, preferably Phe;

Phe may be replaced with His, Tyr, or Trp, preferably His or Tyr;

Tyr may be replaced with His, Phe or Trp, preferably Phe or Trp;

Trp may be replaced with Phe or Tyr, preferably Tyr;

Asn may be replaced with Gln or Ser, preferably Gln;

Gln may be replaced with His, Lys, Glu, Asn, or Ser, preferably Asn or Ser;

Ser may be replaced with Gln, Thr, Pro, Cys, or Ala;

Thr may be replaced with Gln or Ser, preferably Ser;

Lys may be replaced with Gln or Arg;

Arg may be replaced with Lys, Asp or Glu, preferably Lys or Asp;

Asp may be replaced with Lys, Arg, or Glu, preferably Arg or Glu; and

Glu may be replaced with Arg or Asp, preferably Asp.

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Once made, changes can be routinely screened to determine their effects on antigenicity with antibodies which bind to the antigen.

The term "antigenic equivalents" as used herein, refers to proteins or peptides which bind to an antibody which binds to the protein or peptide with which equivalency is sought to be established. Antibodies which are used to select such antigenic equivalents are referred to as "selection antibodies" herein. Antigenic equivalents may be formed by modifying reactive groups within a natural sequence or modifying the N-terminal amino and/or C-terminal carboxyl group. Such equivalents include salts formed with acids and/or bases, particularly physiologically acceptable inorganic and organic acids and bases. Other equivalents include modified carboxyl and/or amino groups on the antigen to produce esters or amides, or amino acid protecting groups such a N-t-butoxycarbonyl. Preferred modifications are those which provide a more stable, active peptide which will be less prone to enzymatic degradation in vivo.

C. Immunocontraceptive Methods

As noted above, the present invention provides an immunocontraceptive method comprising administering an animal subject an antigen as described above in an amount effective to reduce the fertility of that subject. Partial reductions in fertility (i.e., effects which are reflected as a reduction in fertility in a population of subjects) are intended as within the scope of the present invention.

Any animal may be treated by the immunocontraceptive method of the present invention, including both birds and mammals. Exemplary mammals include mice, rabbits, dogs, cats, cows, pigs, sheep, horses and humans. Mammalian subjects are preferred. The subject may be male or female. The antigen may be administered to the subject by any suitable means. Exemplary are by intramuscular injection, by subcutaneous

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injection, by intravenous injection, by intraperitoneal injection, by oral administration, and by nasal spray.

The amount of antigen administered will depend upon factors such as route of administration, species, and the use of booster administrations. In general, a dosage of about 0.1 to about 100 μg per pound subject body weight may be used, more particularly about 1 μg per pound.

The immunocontraceptive method of the present invention contemplating both human and veterinary treatments, the antigens of the present invention may be prepared as both human and veterinary vaccine formulations. Vaccine formulations of the present invention comprise the antigen in a pharmaceutically acceptable carrier. The antigen is included in the carrier in an amount effective to reduce the fertility of the subject being treated. Pharmaceutically acceptable carriers are preferably liquid, particularly aqueous, carriers, such as sodium phosphate buffered saline. The vaccine formulation may be stored in a sterile glass container sealed with a rubber stopper through which liquids may be injected and formulations withdrawn by syringe.

Vaccine formulations of the present invention may optionally contain one or more adjuvants. Any suitable adjuvant can be used, exemplary being aluminum hydroxide, aluminium phosphate, plant and animal oils, and the like, with the amount of adjuvant depending on the nature of the particular adjuvant employed. In addition, the vaccine formulations may also contain one or more stabilizer, exemplary being carbohydrates such as sorbitol, mannitol, starch, sucrose, dextrin, and glucose, proteins such as albumin or casein, and buffers such as alkaline metal phosphate and the like.

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D. Diagnostic Methods

The diagnostic methods of the present invention provide a method of diagnosing autoimmune infertility in both male and female subjects. The term "autoimmune" is here used in a generic sense, as the immunity in female subjects is to exogenous sperm.

Any conventional procedure for detecting antibodies can be employed in practicing the diagnostic assay of the present invention, including agglutination and precipitation reactions, radioimmunoassays, enzyme immunoassays (e.g., U.S. Pat. No. 3,654,090) such as Enzyme-Linked Immunosorbent Assays (ELISA), heterogeneous fluorescent immunoassays (e.g., U.S. Pat. Nos. 4,201,763; 4,171,311; and 3,992,631), and homogeneous (separation-free) immunoassays. See generally *Basic and Clinical Immunology*, 364-73 (J. Fudenberg et al., eds. 3d Ed. 1980), ELISA is preferred.

In a preferred embodiment, serum from a human to be diagnosed is contacted with an antigen as described above so that antibodies in the serum react in solution with the antigen. While the antigen is preferably bound to a solid support, if a homogeneous (separation free) immunoassay is utilized to detect the antibodies, a solid support would not be required.

Serum may be obtained from a person generally pricking a finger and obtaining whole blood (of which serum is a constituent). However, the blood may be processed to obtain only the serum or plasma portion of the whole blood before contacting the serum with the bound antigens. Any method for obtaining serum or plasma from a patient may be utilized as long as the antibodies contained therein retain their ability to bind the antigen.

The antigens may be bound to solid supports by known techniques. For example, a bi-functional organic molecule may be used to attach the antigen to a solid support. The solid can be made of materials such as

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plastic (e.g., the bottom surface of a well in a microtiter plate), fiberglass, cellulose acetate and nitrocellulose (e.g., discs). After being attached or adhered to the solid support, the antigens can be cross-linked if desired.

The step of contacting the solid support with a detectable antibody is carried out so that the detectable antibody is allowed to interact with the antigen bound to the solid support. The detectable antibody is one which is capable of binding to a human antibody from the serum of the patient which has bound to the purified antigen, where the detectable antibody is capable of being detected. More particularly, the detectable antibody can be an anti-human immunoglobulin which is conjugated to a group such as an enzyme which is detectable in the presence of a substrate. Enzyme-conjugated goat or rabbit anti-human antibodies which have been affinity purified are preferred. In general, the detectable group which is conjugated to the detectable antibody may be any enzyme or other detectable species which has been developed for immunoassays. For example, enzymes, fluorescent groups, radioactive groups and others could be used. The enzyme peroxidase is particularly preferred. When peroxidase is the detectable group conjugated to the detectable antibody, a substrate such as 3,3', 5,5'-tetramethylbenzidine or o-phenylenediamine may be used as the substrate for detection of the detectable antibody.

The step of detecting the detectable antibody that has reacted with the human antibodies involves treating or manipulating the detectable group which is conjugated to the detectable antibody to determine its presence. For example, if an enzyme such as peroxidase is conjugated to the antibody, the detecting step would involve adding a peroxidase substrate to the bound antibody, and adding a peroxidase substrate to the bound antibody and observing a color change as peroxidase

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catalyzes conversion of the substrate to a colored species. In the case of other enzymes, such as alkaline phosphatase and β -D-galactosidase, other substrates may be used. The substrate to be used should be chosen such
5 that after the enzyme catalyzes a chemical conversion of the substrate to a product, a change which is observable to a person employing this test should result. Substrates such as 3.3', 5.5'-terramethylbenzidine, p-nitrophenyl phosphate or 3.3'-diamino-benzidine may be
10 used as substrates. Other detectable groups may also be conjugated to the antibody.

A kit containing the required components for carrying out a diagnostic test based on detection of serum antibodies can be assembled. The kit comprises a
15 package containing purified antigen coated in or on a solid support such as the bottom of a microtiter plate well or a nitrocellulose or cellulose acetate disc, and a container of a detectable antibody conjugate which is capable of binding antibody from the serum of a patient
20 which is bound to the antigen. An ELISA test is most preferred for the kit since it lends itself to a readily detectable positive or negative diagnosis. Thus, the kit should also house a container of a substrate which is reactive with an enzyme which is conjugated to the
25 detectable antibody, the substrate being readily detectable after reaction with the enzyme. The antigen employed in the diagnostic kit is preferably substantially or essentially free of other proteins.

E. Avirulent Carrier Cells

30 As noted above, avirulent carrier cells such as microbes are used to administer antigens of the present invention. This method is particularly suitable since appropriate carrier microbes can stimulate production of sIgA to the antigens which they express. Suitable
35 avirulent carrier cells, including both plant carrier cells and microbial carrier cells, are described in R. Curtiss, Vaccines Obtained from Antigenic Gene Products

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of Recombinant Genes, U.S. Patent No. 4,888,170, R. Curtiss and G. Cardineau, Oral Immunization by Transgenic Plants, PCT Application WO 90/02484, and R. Curtiss, Recombinant Avirulent Salmonella Antifertility Vaccines, 5 PCT Application WO 92/09684, the disclosures of which are incorporated herein by reference.

In general, recombinant plasmids containing one or more genes for the gamete-specific antigens can be introduced into one of several avirulent strains of 10 bacteria containing mutations for genes necessary for long-term survival in the targeted host. Useful avirulent microbes include, but are not limited to, mutant derivatives of Salmonella and E. coli-Salmonella hybrids. Preferred microbes are members of the genus 15 Salmonella such as S. typhimurium, S. typhi, S. paratyphi, S. gallinarum, S. pullorum, S. enteritidis, S. choleraesuis, S. arizona, or S. dublin. Avirulent derivatives of S. typhimurium and S. enteritidis find broad use among many hosts. Avirulent derivatives of S. 20 gallinarum, S. pullorum and S. arizona may be particularly useful for immunizing avian species whereas S. typhimurium, S. typhi and S. paratyphi are preferred for use in humans. S. choleraesuis is preferably used to immunize swine while S. dublin finds use in cattle.

25 Particularly useful are one, two or all three of the cya, crp and asd mutants which are substantially incapable of producing the corresponding functional protein in a host, such that growth is impaired. However, other avirulent microbes will also find use with 30 the present invention. Such avirulent microbes include those with aroA, aroD, galE, phoP, cdt, omr and htrA mutations. If Asd mutants are used, the antigen of interest is transferred to the carrier microbe using a vector encoding both the antigen and asd. Thus, only 35 those carrier microbes containing the desired gamete-specific antigen will survive and these microbes can be selected for further use. Expression of the recombinant

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gene encoding the desired antigen maybe dependent on a control sequence linked to the asd gene. This linkage may result from the orientation of the two genes in the vector so that both genes could be, for example, under
5 the control of the same control elements, i.e., the same promoter and operator.

The cya mutants and/or crp mutants can be further mutated, preferably by a deletion, in a gene adjacent to the crp gene which governs virulence of
10 Salmonella. Mutation in this gene, the cdt gene, diminishes the ability of the bacteria to effectively colonize deep tissues, e.g., the spleen. When a plasmid having the crp⁺ gene is placed in a strain with the $\Delta(\text{crp-cdt})$, it retains its avirulence and immunogenicity
15 thus having a phenotype similar to cya and crp mutants. Mutants with the $\Delta(\text{crp-cdt})$ mutation containing a crp⁺ gene on a plasmid retain the normal ability to colonize the intestinal tract and GALT, but have a diminished ability to colonize deeper tissues.

20 In order to stimulate a preferred immune response, introduction of the microbe or gene product directly into the gut or bronchus is preferred, such as by oral administration, intranasal administration, gastric intubation or in the form of aerosols, as well as
25 air sac inoculation (in birds only), and intratracheal inoculation. Other suitable methods include administration via the conjunctiva to reach the Harder gland and intramammary inoculation. Other methods of administering the vaccine, such as intravenous,
30 intramuscular, or subcutaneous injection are also possible, and used principally to stimulate a secondary immune response, as described further below.

Generally, when carrier microbes expressing the antigens are administered to humans or other mammals,
35 they will be present in a pharmaceutically acceptable carrier. For example, the carrier microbes can be enteric-coated or encapsulated with a suitable gelatin-

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like substance, known in the art (Cryz and Glück, 1990, in G. Woodrow and Mr. Levine, New Generation Vaccines, Marcel Dekker, New York, pp. 921-932).

Once the carrier microbe is present in the animal, the antigen must become available to the animal's immune system. This may be accomplished when the carrier microbe dies so that the antigen molecules are released. Of course, the use of "leaky" avirulent mutants that release the contents of the periplasm without lysis is also possible. Alternatively, a gene may be selected that controls the production of an antigen that will be made available by the carrier cell to the outside environment prior to the death of the cell.

The antigens may also be administered as aerosols or intranasally. Intranasal formulations for human subjects will usually include vehicles that neither cause irritation to the nasal mucosa nor significantly disturb ciliary function. Diluents such as water, aqueous saline or other known substances can be employed with the subject invention. The nasal formulations may also contain preservatives such as but not limited to chlorobutanol and benzalkonium chloride. A surfactant may be present to enhance absorption of the subject proteins by the nasal mucosa.

Injection of the gamete-specific antigen can also be done in conjunction with prior oral, intranasal, gastric or aerosol immunization. Such parenteral immunization can serve as a booster to enhance expression of the secretory immune response once the secretory immune system to the gamete-specific gene product has been primed by immunization with the carrier microbe expressing the gamete-specific gene product. The enhanced response is known as a secondary, booster, or anamnestic response and results in prolonged immune protection of the host. Booster immunizations may be repeated numerous times with beneficial results.

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When the vaccines are prepared as injectables, such as for boosters, they can be made either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid vehicles prior to injection may also be prepared. The preparation may also be emulsified or the active ingredient encapsulated in liposome vehicles. The active immunogenic ingredient is often mixed with vehicles containing excipients which are pharmaceutically acceptable and compatible with the active ingredient. Suitable vehicles are, for example, water, saline, dextrose, glycerol, ethanol, or the like, and combinations thereof. In addition, if desired, the vehicle may contain minor amounts of auxiliary substances such as wetting or emulsifying agents, pH buffering agents, or adjuvants which enhance the effectiveness of the vaccine. Adjuvants may include for example, muramyl dipeptides, avridine, aluminum hydroxide, oils, saponins and other substances known in the art. Actual methods of preparing such dosage forms are known, or will be apparent, to those skilled in the art. See, e.g., Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, PA, 15th ed., 1975. The composition or formulation to be administered will, in any event, contain a quantity of the protein adequate to achieve the desired immunized state in the individual being treated.

The quantity of antigen to be administered depends on the subject to be treated, the capacity of the subject's immune system to synthesize antibodies, and the degree of protection desired. Effective dosages can be readily established by one of ordinary skill in the art through routine trials establishing dose response curves. The subject is immunized by administration of the particular antigen or fragment thereof, or analog thereof, in at least one dose. Typical doses using the carrier microbe are on the order of 1×10^6 - 1×10^{10} recombinant avirulent bacteria/immunized subject. The subject may be administered increasing amounts or

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multiple dosages as required to maintain a state of immunity to the gamete-specific antigen.

It may be desirable to administer more than one antigen simultaneously or consecutively. This can be accomplished either by administering an avirulent carrier containing genes encoding for more than one gamete-specific antigen or by administering different carrier organisms.

The present invention is explained below in the following non-limiting Examples.

EXAMPLE 1

Cloning and Sequencing of Human

Sperm Zona Binding Protein Sp17

We have previously reported cloning and sequence data of rabbit Sp17 (Richardson and O'Rand, *Mol. Biol. Cell* 3, 15a (1992)). This protein is known to be a member of the rabbit sperm antigen (RSA) family of rabbit testis/sperm autoantigens and also to be expressed in mice. Further search for a human counterpart of this protein was initiated with screening of a human testis cDNA library using the protein coding region of the rabbit Sp17 gene as a probe. One clone contained a 1287 base pair insert 71% identical to the rabbit Sp17 gene at the nucleotide level, contained an open reading frame of 455 base pairs, and had the sequence given herein as SEQ ID NO:1. This clone encoded a protein of 151 amino acids having the sequence given herein as SEQ ID NO:2 with a calculated molecular weight of 17,534 Da, 76.7% identical to the rabbit Sp17 and 71.8% identical to the mouse Sp17 protein sequence. In particular, the first 44 amino acids are completely identical in the mouse, rabbit and human sequences and have a 43% identity to the human testis cAMP dependent protein kinase type II α regulatory sub-unit dimer interaction site. Interestingly, comparison of rabbit, mouse and human amino acid sequences has shown that the human Sp17 lacks

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the single cysteine residue at the center of the molecule which the other sequences possess. Northern blot analysis of a range of mouse, baboon and human tissues revealed a highly restricted pattern of gene expression limited to the testis. Additionally, Northern analysis has revealed evidence of 2 distinct transcript sizes of approximately 1.3 kb and 0.9 kb in the human testis. Antisera to Sp17 recombinant protein (rSp17) has been generated and used on Western blots of human sperm lysates to demonstrate a predominant immunoreactive protein of 29 KDa. The antigen was localized by immunofluorescence on human spermatozoa with anti-rSp17. In ELISA rSp17 was shown to bind fucoidan with saturation kinetics. Sera from vasectomized men who have anti-sperm antibody titres also shown reactivity to rSp17, indicating that human Sp17 is a human sperm autoantigen.

EXAMPLE 2

Production of Sequential Decapeptides

20 and Immunoassay Procedures

A series of forty-seven N-terminal acetylated sequential decapeptides corresponding to fragments of the rabbit Sp17 protein were synthesized in accordance with known techniques; these decapeptides are disclosed herein as SEQ ID NO:3 through SEQ ID NO:49.

Enzyme-linked immunosorbent assay (ELISA) was carried out in accordance with known procedures (see, e.g., M. O'Rand et. al. *Dev. Biol.* **129**, 231 (1988)), as adapted for the MULTIPIN™ system, in accordance with the manufacturer's specifications. (Chiron Mimotopes Pty. Ltd., Clayton, Victoria, 3168 Australia). Control O.D. values of IgG from a normal control subject were subtracted from experimental values in displaying the ELISA data below, in accordance with standard techniques.

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EXAMPLE 3**Production of Antibodies to Peptide G22C**

The peptide G22C, which corresponds to the fragment of rabbit Sp17 spanning amino acid 61 to amino acid 82 (and having the sequence: GAKVDDRFYNNHAFQEHSEK) was synthesized at the Salk Institute under contract NO1-HD-0-2906 with the NIH in accordance with standard techniques.

Male and female rabbits were immunized with G22C peptide which was conjugated to keyhole limpet hemocyanin (KLH) with the C-terminal Cys amino acid of G22C. Conjugation was carried out with Ellman's reagent, 5,5'-dithio-bis-[2-nitrobenzoic acid] obtained from Pierce Scientific in the form of the IMJECT® immunogen conjugation kit. Each rabbit received subcutaneous injection of 300 µg of conjugate in complete Freund's adjuvant followed by an additional 200 µg of conjugate in incomplete Freund's adjuvant three weeks later and a final 100 µg of conjugate in incomplete Freund's adjuvant three weeks later. Conjugate was provided in 100 µl of water diluted 1:1 with adjuvant.

EXAMPLE 4**Binding of Rabbit AutoImmune Sera to
Rabbit Sp17 Sequential Decapeptides**

A male rabbit was injected with his own sperm to produce autoimmune sera. Specifically, 2 mg of sperm was washed three times in PBS, resuspended in .5 ml of PBS, diluted 1:1 by volume with Freund's complete adjuvant, and injected subcutaneously. A first booster shot was given one month thereafter, a second booster was given an additional two weeks thereafter, a third booster was given an additional two weeks thereafter, and a fourth booster was given an additional three months thereafter. The immune sera was screened by ELISA as described above with the sequential decapeptides

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described above. Results are shown in Figure 1. Note the clustering of potential autoantigenic epitopes.

EXAMPLE 5

Binding of Pooled Human AutoImmune Sera to

5 Rabbit Sp17 Sequential Decapeptides

Figure 2 shows the binding of a pool of equal volumes of sera from four vasectomized men with high titers of antisperm antibodies tested against the sequential decapeptides described above by ELISA as described above.

Note that all the peaks on Figure 2 represent human autoantigenic B-cell epitopes.

EXAMPLE 6

Binding of Sp17-Immunized Rabbit Sera to

15 Rabbit Sp17 Sequential Decapeptides

The binding of immune sera from a femal rabbit immunized with recombinant rabbit Sp17 fusion protein to the sequential decapeptides described above is shown in Figure 3.

20 The recombinant fusion protein was generated by PCR using the following primers: for the plus strand, 5'-CGCGGATCCATGTCGATTCCATTTTCC-3' which contains a Bam HI site, and for the antisense primer, 5'-CGGGGTACCGCCAGTGCC-CTCAATTGT-3', which contains a Kpn I
25 site. The PCR product was directionally cloned into the polylinker region of pQE-30, sequenced to verify integrity of the insert, and bacterially expressed according to the protocol provided by Qiagen Inc. (Chatsworth, CA). Using this system, the recombinant
30 rabbit Sp17 protein (rSp17) is expressed minus the first 11 N-terminal amino acids missing but with an N-terminal containing the Sequence Arg-Gly-Ser, followed by six histidines and a glycine which precede the Sp17 amino acids. The fusion protein was purified from the
35 bacterial lysate by affinity chromatography using the metal chelate adsorbent nickel-NTA-agarose (Qiagen,

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Inc.). The fusion protein was eluted with 8 M urea, 0.1 M sodium phosphate (monobasic), 0.01 M Tris, pH adjusted to 5.9, and dialyzed against three changes of PBS. 200 µg of protein in .5 ml PBS with 1 mg ADJUPRIME™ adjuvant (Pierce Chemical Co., Rockford, Illinois, USA) was administered subcutaneously. A first booster of 200 µg in the same volume was given three weeks thereafter.

EXAMPLE 7

Binding of G22C-Immunized Rabbit Sera to

10 Rabbit Sp17 Sequential Decapeptides

The binding of immune sera taken from a male rabbit immunized with the synthetic peptide G22C as described in Example 3 above to the sequential decapeptides described above by ELISA as described above is shown in Figure 4, and the binding of immune sera taken from a female rabbit immunized with the synthetic peptide G22C as described in Example 3 above to the sequential decapeptides described above is shown in Figure 5.

20

EXAMPLE 8

Binding of Human Sp17 Rabbit Immune Sera to

Rabbit Sp17 Sequential Decapeptides

Figure 7 illustrates the effect of immunization of mice with recombinant human Sp17 (fusion protein) on fertility. Six mice received only mouse Sp17, six received mouse and then rabbit Sp17, six received only human Sp17. Adjuvant controls (n=12) received TITERMAX™ adjuvant (available from Sigma Co., St. Louis) only. Six mice received no injections. The mice immunized with the human Sp17 recombinant protein showed a 42% decrease in pregnancy.

Recombinant human Sp17 was prepared as a fusion protein in essentially the same manner as described above for recombinant rabbit Sp17, except that no N-terminal Sp17 amino acids were deleted from the resulting product.

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Balb/c mice were immunized with approximately 5 μ g of fusion protein in water diluted 1:1 with Freund's complete adjuvant. Four weeks after the first immunization, three more injections of the fusion protein in incomplete adjuvant were given every two weeks.

EXAMPLE 9

Alignment of Mammalian Sp17s

Figure 8 gives the alignment of the rabbit (RABSP17), mouse (MUSSP17), and human (HUMSP17) Sp17 protein sequences. Autoantigenic fragments are indicated in the boxes. Numbering is from N-terminus to C-terminus, based on the numbering of the human sequence, with gaps introduced into the other mammalian sequences to maximize alignment of the autoantigenic fragments shown in the boxes, and numbers skipped where gaps are introduced so that the numbering of the autoantigenic fragments indicated in the boxes corresponds across species.

EXAMPLE 10

Binding of Recombinant Sp17 to Human Zona Pellucida

Biotinylated human recombinant Sp17 was shown to bind to human zona pellucida by ELISA. This is the first demonstration of binding of any recombinant mammalian Sp17 to any mammalian zona pellucida.

The foregoing examples are illustrative of the present invention, and are not to be construed as limiting thereof. The invention is defined by the following claims, with equivalents of the claims to be included therein.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: O'Rand, Michael G.
Widgren, Esther E.
Richardson, Richard T.
Lea, Isabel

(ii) TITLE OF INVENTION: Sperm Antigen Corresponding to a Sperm Zona Binding Protein Autoantigenic Epitope

(iii) NUMBER OF SEQUENCES: 49

(iv) CORRESPONDENCE ADDRESS:

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(C) CITY: Charlotte
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(F) ZIP: 28234

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: Sibley, Kenneth D.
(B) REGISTRATION NUMBER: 31,665
(C) REFERENCE/DOCKET NUMBER: 5470/73

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: 919-881-3140
(B) TELEFAX: 919-881-3175

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 854 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

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(iv) ANTI-SENSE: NO

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 32..487

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

| | |
|---|-----|
| GGAGGTTCCA TAGGCAGTTC TTACCAAGAA G ATG TCG ATT CCA TTC TCC AAC | 52 |
| Met Ser Ile Pro Phe Ser Asn | |
| 1 5 | |
| ACC CAC TAC CGA ATT CCA CAA GGA TTT GGG AAT CTT CTT GAA GGG CTG | 100 |
| Thr His Tyr Arg Ile Pro Gln Gly Phe Gly Asn Leu Leu Glu Gly Leu | |
| 10 15 20 | |
| ACA CGC GAG ATT CTG AGA GAG CAA CCG GAC AAT ATA CCA GCT TTT GCA | 148 |
| Thr Arg Glu Ile Leu Arg Glu Gln Pro Asp Asn Ile Pro Ala Phe Ala | |
| 25 30 35 | |
| GCA GCC TAT TTT GAG AGC CTT CTA GAG AAA AGA GAG AAA ACC AAC TTT | 196 |
| Ala Ala Tyr Phe Glu Ser Leu Leu Glu Lys Arg Glu Lys Thr Asn Phe | |
| 40 45 50 55 | |
| GAT CCA GCA GAA TGG GGG AGT AAG GTA GAA GAC CGC TTC TAT AAC AAT | 244 |
| Asp Pro Ala Glu Trp Gly Ser Lys Val Glu Asp Arg Phe Tyr Asn Asn | |
| 60 65 70 | |
| CAT GCA TTC GAG GAG CAA GAA CCA CCT GAG AAA AGT GAT CCT AAA CAA | 292 |
| His Ala Phe Glu Glu Gln Glu Pro Pro Glu Lys Ser Asp Pro Lys Gln | |
| 75 80 85 | |
| GAA GAG TCT CAG ATA TCT GGG AAG GAG GAA GAG ACA TCA GTC ACC ATC | 340 |
| Glu Glu Ser Gln Ile Ser Gly Lys Glu Glu Glu Thr Ser Val Thr Ile | |
| 90 95 100 | |
| TTA GAC TCT TCT GAG GAA GAT AAG GAA AAA GAA GAG GTT GCT GCT GTC | 388 |
| Leu Asp Ser Ser Glu Glu Asp Lys Glu Lys Glu Glu Val Ala Ala Val | |
| 105 110 115 | |
| AAA ATC CAA GCT GCC TTC CGG GGA CAC ATA GCC AGA GAG GAG GCA AAG | 436 |
| Lys Ile Gln Ala Ala Phe Arg Gly His Ile Ala Arg Glu Glu Ala Lys | |
| 120 125 130 135 | |
| AAA ATG AAA ACA AAT AGT CTT CAA AAT GAG GAA AAA GAG GAA AAC AAG | 484 |
| Lys Met Lys Thr Asn Ser Leu Gln Asn Glu Glu Lys Glu Glu Asn Lys | |
| 140 145 150 | |
| TGAGGACACT GGTTTTACCT CCAGGAAACA TGAAAAATAA TCCAAATCCA TCCATCAACC | 544 |
| TTCTTATTAA TGTCATTTCT CCTTGAGGAA GGAAGATTG ATGTTGTGAA ATAACATTG | 604 |
| TTACTGTTGT GAAAATCTGT CATGAGCATT TGTTTAATAA GCATACCATT GAAACATGCC | 664 |

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ACTTGAAGAT TTCTCTGAGA TCATGAGTTT GTTTACACTT GTCTCAAGCC TATCTATAGA 724
 GACCCCTTGA TTTAGAATTA TAGAACTAAA GTATCTGAGA TTACAGAGAT CTCAGAGGTT 784
 ATGTGTTCTA ACTATTATCA AATGAATAAA TCCTCTCTAT CACATCCCCC AAAAAAAAAA 844
 AAAAAAAAAA 854

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 151 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Ser Ile Pro Phe Ser Asn Thr His Tyr Arg Ile Pro Gln Gly Phe
 1 5 10 15
 Gly Asn Leu Leu Glu Gly Leu Thr Arg Glu Ile Leu Arg Glu Gln Pro
 20 25 30
 Asp Asn Ile Pro Ala Phe Ala Ala Tyr Phe Glu Ser Leu Leu Glu
 35 40 45
 Lys Arg Glu Lys Thr Asn Phe Asp Pro Ala Glu Trp Gly Ser Lys Val
 50 55 60
 Glu Asp Arg Phe Tyr Asn Asn His Ala Phe Glu Glu Gln Glu Pro Pro
 65 70 75 80
 Glu Lys Ser Asp Pro Lys Gln Glu Glu Ser Gln Ile Ser Gly Lys Glu
 85 90 95
 Glu Glu Thr Ser Val Thr Ile Leu Asp Ser Ser Glu Glu Asp Lys Glu
 100 105 110
 Lys Glu Glu Val Ala Ala Val Lys Ile Gln Ala Ala Phe Arg Gly His
 115 120 125
 Ile Ala Arg Glu Glu Ala Lys Lys Met Lys Thr Asn Ser Leu Gln Asn
 130 135 140
 Glu Glu Lys Glu Glu Asn Lys
 145 150

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 10 amino acids

(B) TYPE: amino acid

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(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

| | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Met | Ser | Ile | Pro | Phe | Ser | Asn | Thr | His | Tyr |
| 1 | | | 5 | | | | | | 10 |

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

| | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Pro | Phe | Ser | Asn | Thr | His | Tyr | Arg | Ile | Pro |
| 1 | | | 5 | | | | | | 10 |

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

| | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Asn | Thr | His | Tyr | Arg | Ile | Pro | Gln | Gly | Phe |
| 1 | | | 5 | | | | | | 10 |

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

| | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Tyr | Arg | Ile | Pro | Gln | Gly | Phe | Gly | Asn | Leu |
| 1 | | | | 5 | | | | | 10 |

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 10 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

| | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Pro | Gln | Gly | Phe | Gly | Asn | Leu | Leu | Glu | Gly |
| 1 | | | | 5 | | | | | 10 |

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 10 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

| | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Phe | Gly | Asn | Leu | Leu | Glu | Gly | Leu | Thr | Arg |
| 1 | | | | 5 | | | | | 10 |

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 10 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

| | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Leu | Leu | Glu | Gly | Leu | Thr | Arg | Glu | Ile | Leu |
| 1 | | | | 5 | | | | | 10 |

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(2) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 10 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

| | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Gly | Leu | Thr | Arg | Glu | Ile | Leu | Arg | Glu | Gln |
| 1 | | | | 5 | | | | | 10 |

(2) INFORMATION FOR SEQ ID NO:11:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 10 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

| | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Arg | Glu | Ile | Leu | Arg | Glu | Gln | Pro | Asp | Asn |
| 1 | | | | 5 | | | | | 10 |

(2) INFORMATION FOR SEQ ID NO:12:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 10 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

| | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Leu | Arg | Glu | Gln | Pro | Asp | Asn | Ile | Pro | Ala |
| 1 | | | | 5 | | | | | 10 |

(2) INFORMATION FOR SEQ ID NO:13:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 10 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

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Gln Pro Asp Asn Ile Pro Ala Phe Ala Ala
1 5 10

(2) INFORMATION FOR SEQ ID NO:14:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Asn Ile Pro Ala Phe Ala Ala Ala Tyr Phe
1 5 10

(2) INFORMATION FOR SEQ ID NO:15:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Ala Phe Ala Ala Ala Tyr Phe Glu Asn Leu
1 5 10

(2) INFORMATION FOR SEQ ID NO:16:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Ala Ala Tyr Phe Glu Asn Leu Leu Glu Lys
1 5 10

(2) INFORMATION FOR SEQ ID NO:17:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Phe Glu Asn Leu Leu Glu Lys Arg Glu Lys
1 5 10

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 10 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Leu Leu Glu Lys Arg Glu Lys Thr Asn Phe
1 5 10

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 10 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Lys Arg Glu Lys Thr Asn Phe Asp Pro Ala
1 5 10

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 10 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Lys Thr Asn Phe Asp Pro Ala Glu Trp Gly
1 5 10

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 10 amino acids

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- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

Phe Asp Pro Ala Glu Trp Gly Ala Lys Val
1 5 10

(2) INFORMATION FOR SEQ ID NO:22:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 10 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

Ala Glu Trp Gly Ala Lys Val Asp Asp Arg
1 5 10

(2) INFORMATION FOR SEQ ID NO:23:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 10 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

Gly Ala Lys Val Asp Asp Arg Phe Tyr Asn
1 5 10

(2) INFORMATION FOR SEQ ID NO:24:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 10 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

Val Asp Asp Arg Phe Tyr Asn Asn His Ala
1 5 10

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(2) INFORMATION FOR SEQ ID NO:25:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 10 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

| | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Arg | Phe | Tyr | Asn | Asn | His | Ala | Phe | Gln | Glu |
| 1 | | | | 5 | | | | | 10 |

(2) INFORMATION FOR SEQ ID NO:26:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 10 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

| | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Asn | Asn | His | Ala | Phe | Gln | Glu | His | Glu | Ser |
| 1 | | | | 5 | | | | | 10 |

(2) INFORMATION FOR SEQ ID NO:27:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 10 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

| | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Ala | Phe | Gln | Glu | His | Glu | Ser | Glu | Lys | Cys |
| 1 | | | | 5 | | | | | 10 |

(2) INFORMATION FOR SEQ ID NO:28:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 10 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

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Glu His Glu Ser Glu Lys Cys Glu Ala Glu
1 5 10

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 10 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

Ser Glu Lys Cys Glu Ala Glu Glu Lys Ser
1 5 10

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 10 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

Cys Glu Ala Glu Glu Lys Ser Gln Ser Val
1 5 10

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 10 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

Glu Glu Lys Ser Gln Ser Val Thr Glu Glu
1 5 10

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 10 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

| | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Ser | Gln | Ser | Val | Thr | Glu | Glu | Glu | Thr | Pro |
| 1 | | | | 5 | | | | | 10 |

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 10 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

| | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Val | Thr | Glu | Glu | Glu | Thr | Pro | Val | Leu | Thr |
| 1 | | | | 5 | | | | | 10 |

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 10 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

| | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Glu | Glu | Thr | Pro | Val | Leu | Thr | Ile | Asp | Ser |
| 1 | | | | 5 | | | | | 10 |

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 10 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

| | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Pro | Val | Leu | Thr | Ile | Asp | Ser | Glu | Asp | Asp |
| 1 | | | | 5 | | | | | 10 |

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 10 amino acids

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(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

Thr Ile Asp Ser Glu Asp Asp Lys Asp Lys
1 5 10

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

Ser Glu Asp Asp Lys Asp Lys Glu Glu Met
1 5 10

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

Asp Lys Asp Lys Glu Glu Met Ala Ala Leu
1 5 10

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

Lys Glu Glu Met Ala Ala Leu Lys Ile Gln
1 5 10

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(2) INFORMATION FOR SEQ ID NO:40:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 10 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

Met Ala Ala Leu Lys Ile Gln Ala Ala Phe
1 5 10

(2) INFORMATION FOR SEQ ID NO:41:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 10 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

Leu Lys Ile Gln Ala Ala Phe Arg Gly His
1 5 10

(2) INFORMATION FOR SEQ ID NO:42:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 10 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

Gln Ala Ala Phe Arg Gly His Leu Ala Arg
1 5 10

(2) INFORMATION FOR SEQ ID NO:43:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 10 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

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Phe Arg Gly His Leu Ala Arg Glu Asp Val
1 5 10

(2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 10 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

His Leu Ala Arg Glu Asp Val Lys Lys Ile
1 5 10

(2) INFORMATION FOR SEQ ID NO:45:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 10 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

Arg Glu Asp Val Lys Lys Ile Arg Thr Asn
1 5 10

(2) INFORMATION FOR SEQ ID NO:46:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 10 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

Val Lys Lys Ile Arg Thr Asn Lys Ala Glu
1 5 10

(2) INFORMATION FOR SEQ ID NO:47:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 10 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

| | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Ile | Arg | Thr | Asn | Lys | Ala | Glu | Glu | Glu | Thr |
| 1 | | | | 5 | | | | | 10 |

(2) INFORMATION FOR SEQ ID NO:48:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 10 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

| | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Asn | Lys | Ala | Glu | Glu | Glu | Thr | Glu | Glu | Asn |
| 1 | | | | 5 | | | | | 10 |

(2) INFORMATION FOR SEQ ID NO:49:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 10 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

| | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Lys | Ala | Glu | Glu | Glu | Thr | Glu | Glu | Asn | Asn |
| 1 | | | | 5 | | | | | 10 |

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CLAIMS:

1. Isolated DNA encoding an Sp 17 protein selected from the group consisting of:

5 (a) isolated DNA consisting essentially of DNA which encodes the human Sp 17 protein having the amino acid sequence given herein as SEQ ID NO:2;

10 (b) isolated DNA which hybridizes to isolated DNA of (a) above under conditions represented by a wash stringency of 0.3M NaCl, 0.03M sodium citrate, and 0.1% SDS at 60°C, and which encodes a human Sp 17 protein;

15 (c) isolated DNA differing from the isolated DNAs of (a) and (b) above in nucleotide sequence due to the degeneracy of the genetic code, and which encodes a human Sp 17 protein; and

20 (d) fragments of the foregoing which encode antigenic peptides, which antigenic peptides bind antibodies which bind to the human Sp 17 protein.

2. Isolated DNA according to claim 1 consisting essentially of DNA which encodes the human Sp 17 protein given herein as SEQ ID NO:2.

25 3. Isolated DNA according to claim 1 consisting essentially of isolated DNA having the sequence given herein as SEQ ID NO:1.

4. A recombinant DNA sequence comprising vector DNA and a DNA according to claim 1.

30 5. A host cell containing a recombinant DNA sequence of claim 4 and capable of expressing the encoded protein or peptide.

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6. An immunocontraceptive method, comprising administering to a subject a protein or peptide encoded by a DNA according to claim 1 in an amount effective to reduce the fertility of said subject.

5 7. An immunocontraceptive method according to claim 6, wherein said subject is a female subject.

8. An immunocontraceptive vaccine formulation comprising a protein or peptide encoded by a DNA according to claim 1 in an amount effective to reduce the
10 fertility of a subject in combination with a pharmaceutically acceptable carrier.

9. A method of screening for autoimmune infertility in a subject, comprising:

15 detecting the presence of antibodies in said subject which bind to a protein or peptide encoded by DNA according to claim 1, the presence of said antibodies indicating the subject is afflicted with autoimmune infertility.

20 10. A method according to claim 9, wherein said subject is a male subject.

11. A method according to claim 9, wherein said detecting step comprises detecting the presence of antibodies in said subject which bind to a protein having the sequence given herein as SEQ ID NO:2.

25 12. An avirulent host cell containing a recombinant DNA sequence according to claim 4 and capable of expressing the encoded protein or peptide.

13. An avirulent host cell according to claim 12, which host cell is a microbial host cell.

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14. An immunocontraceptive vaccine formulation comprising an avirulent host cell according to claim 12 in an amount effective to reduce the fertility of a subject in combination with a pharmaceutically acceptable
5 carrier.

15. An antigenic peptide, useful as an immunocontraceptive agent or for the diagnosis of autoimmune infertility, selected from the group consisting of:

10 antigenic fragments of mammalian Sp17 protein which are from six to twenty-five amino acids in length and the antigenic equivalents thereof, said antigenic equivalents being:

(a) a modified fragment comprising said
15 antigenic fragment modified by replacing of one or more amino acids to the sequence thereof; or

(b) a longer peptide which incorporates the sequence of said decapeptide or said modified decapeptide and which has (i) up to four extra amino acid residues
20 attached to the C-terminal end of said sequence, (ii) up to four extra amino acid residues attached to the N-terminal end of said sequence, or (iii) up to four extra amino acid residues attached to the C-terminal end of said sequence and up to four extra amino acid residues
25 attached to the N-terminal end of said sequence;

wherein said antigenic equivalents bind to antibodies which bind to the antibodies which bind to said antigenic fragments of mammalian Sp17 protein;

and wherein said antigenic fragments of
30 mammalian Sp17 protein are selected from the group consisting of (i) amino acid 4 to amino acid 28 fragments, (ii) amino acid 34 to amino acid 49 fragments, (iii) amino acid 55 to amino acid 82 fragments, (iv) amino acid 117 to amino acid 137 fragments, and (v)
35 fragments of (i) through (iv) which are at least six amino acids in length.

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16. An antigenic peptide according to claim 15, wherein said mammalian Sp17 protein is selected from the group consisting of rabbit, mouse, human, baboon, and monkey Sp17 proteins.

5 17. An antigenic peptide according to claim 15 wherein said antigenic fragment is selected from the group consisting of peptides having the amino acid sequence given herein as: SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:34, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:43, and fragments thereof which are at least six amino acids in length.

18. An antigenic peptide according to claim 15 wherein said antigenic fragment is selected from the group consisting of peptides having the amino acid sequence given herein as: SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:43, and fragments thereof which are at least six amino acids in length.

19. An antigenic peptide according to claim 15 wherein said antigenic fragment is selected from the group consisting of peptides having the amino acid sequence given herein as: SEQ ID NO:3, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID

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NO;29, SEQ ID NO;30, SEQ ID NO;32, SEQ ID NO;33, SEQ ID NO;34, SEQ ID NO;35, SEQ ID NO;36, SEQ ID NO;38, SEQ ID NO;39, SEQ ID NO;40, SEQ ID NO;43, SEQ ID NO;47, SEQ ID NO;48, SEQ ID NO;49, and fragments thereof which are at least six amino acids in length.

20. An antigenic peptide according to claim 15 wherein said antigenic fragment is selected from the group consisting of peptides having the amino acid sequence given herein as: SEQ ID NO;8, SEQ ID NO;9, SEQ ID NO;14, SEQ ID NO;15, SEQ ID NO;16, SEQ ID NO;21, SEQ ID NO;22, SEQ ID NO;24, SEQ ID NO;25, SEQ ID NO;34, SEQ ID NO;40, SEQ ID NO;43, and fragments thereof which are at least six amino acids in length.

21. An immunocontraceptive method, comprising administering to a subject peptide according to claim 12 in an amount effective to reduce the fertility of said subject.

22. An immunocontraceptive method according to claim 21, wherein said subject is a female subject.

23. An immunocontraceptive vaccine formulation comprising a peptide according to claim 15 in an amount effective to reduce the fertility of a subject in combination with a pharmaceutically acceptable carrier.

24. A method of screening for autoimmune infertility in a subject, comprising:

detecting the presence of antibodies in said subject which bind to a peptide according to claim 15, the presence of said antibodies indicating the subject is afflicted with autoimmune infertility.

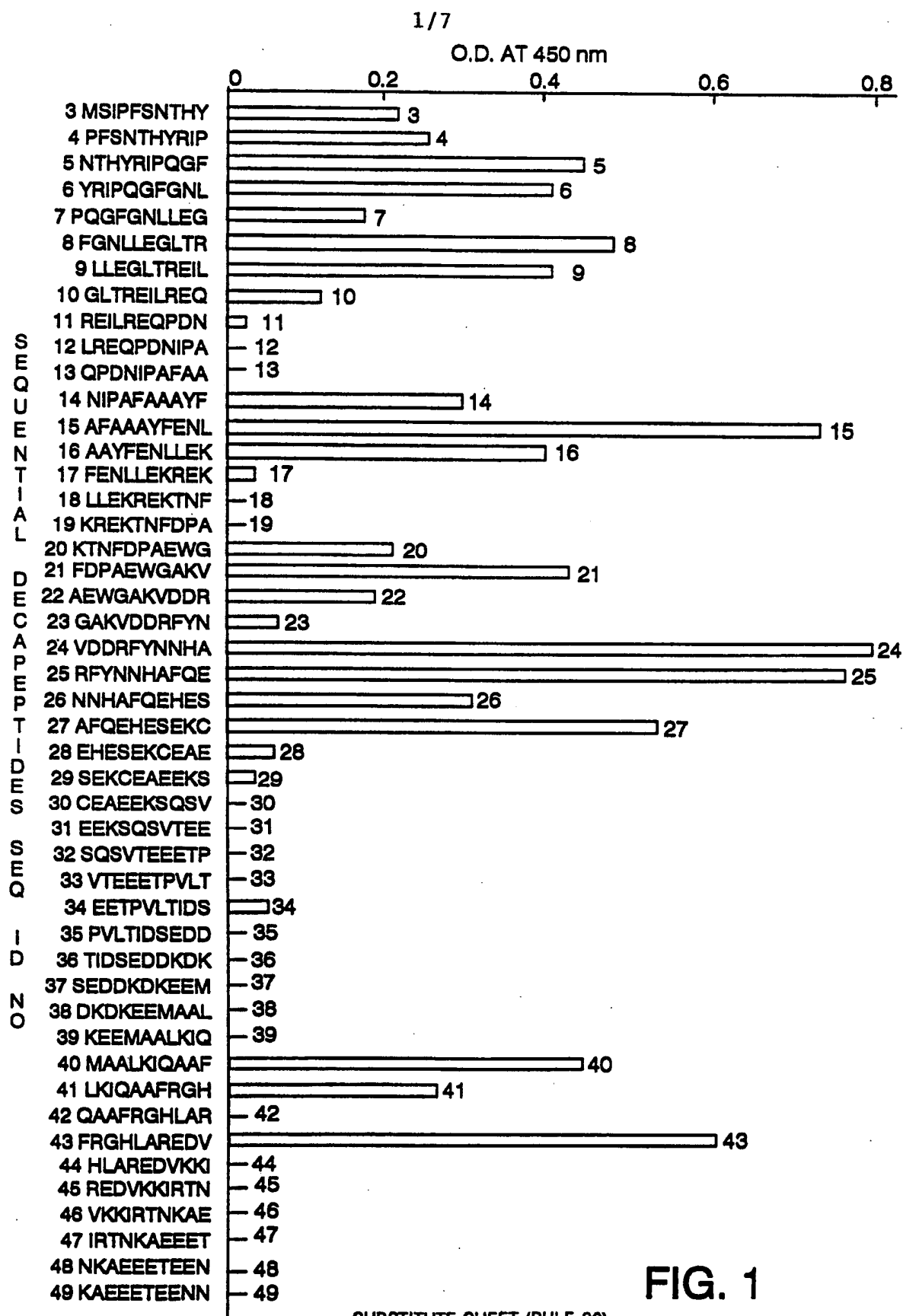
25. A method according to claim 24, wherein said subject is a male subject.

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26. An avirulent host cell containing a recombinant DNA sequence which expresses a peptide according to claim 15.

27. An avirulent host cell according to claim
5 26, which host cell is a microbial host cell.

28. An immunocontraceptive vaccine formulation comprising an avirulent host cell according to claim 26 in an amount effective to reduce the fertility of a subject in combination with a pharmaceutically acceptable
10 carrier.



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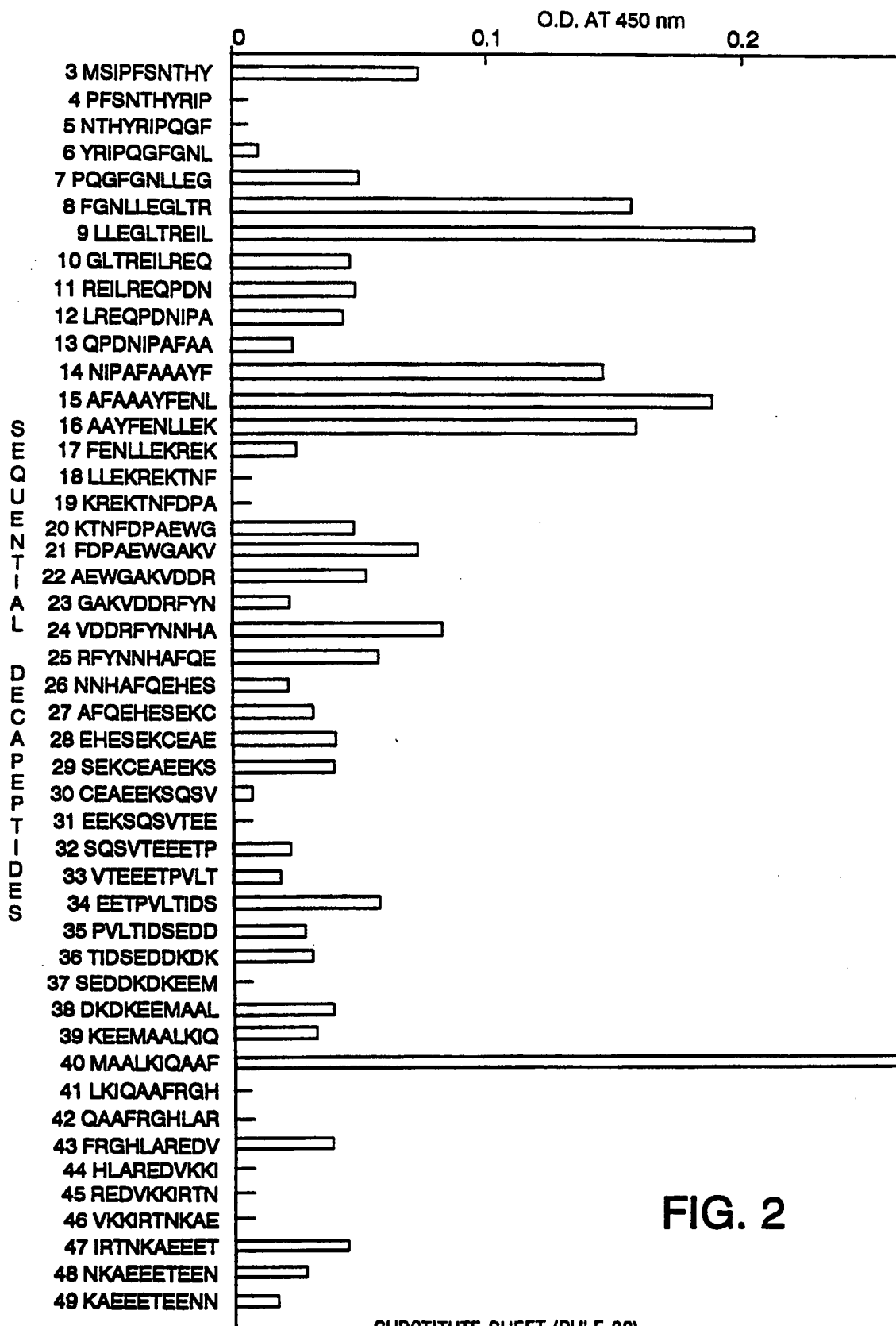


FIG. 2

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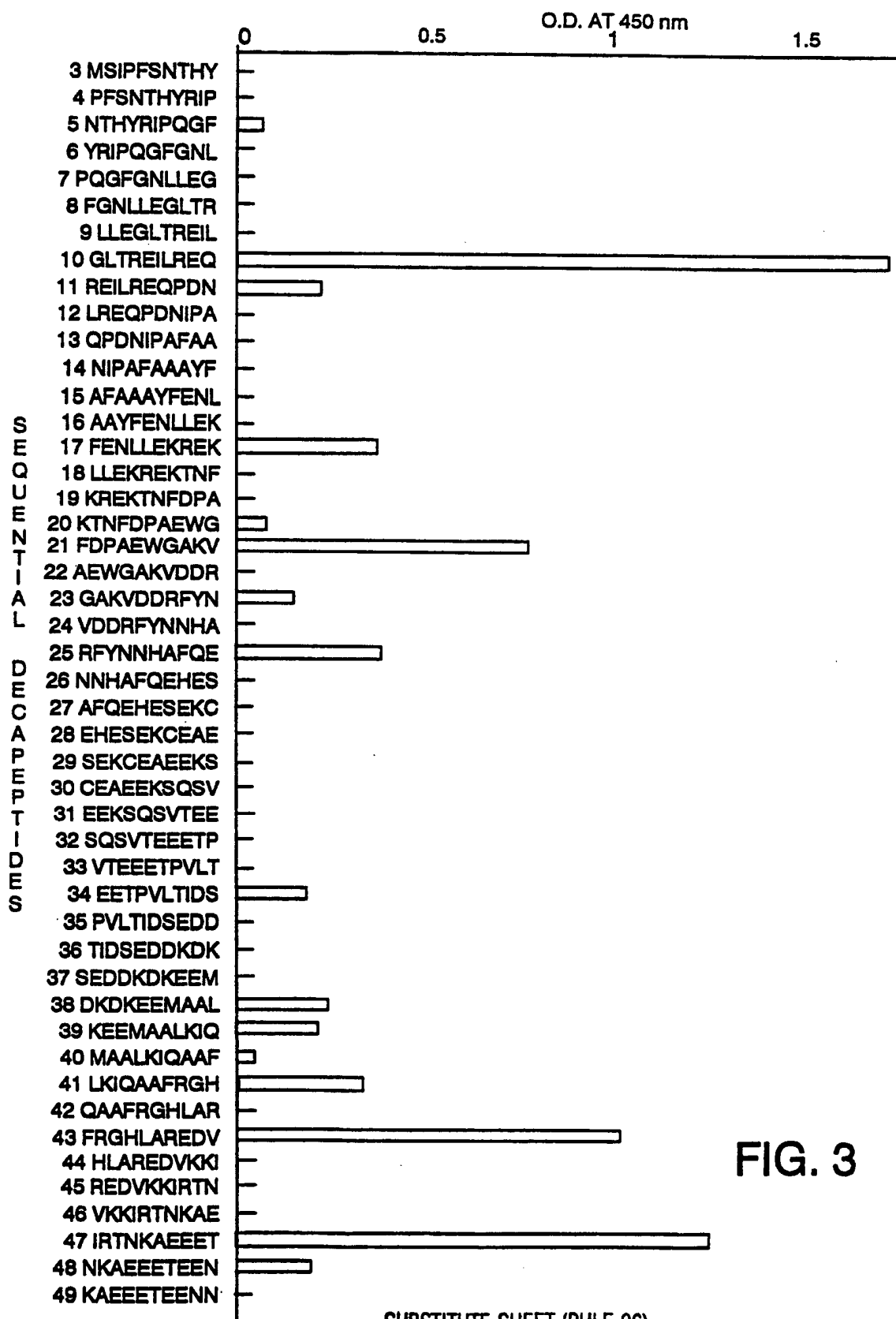
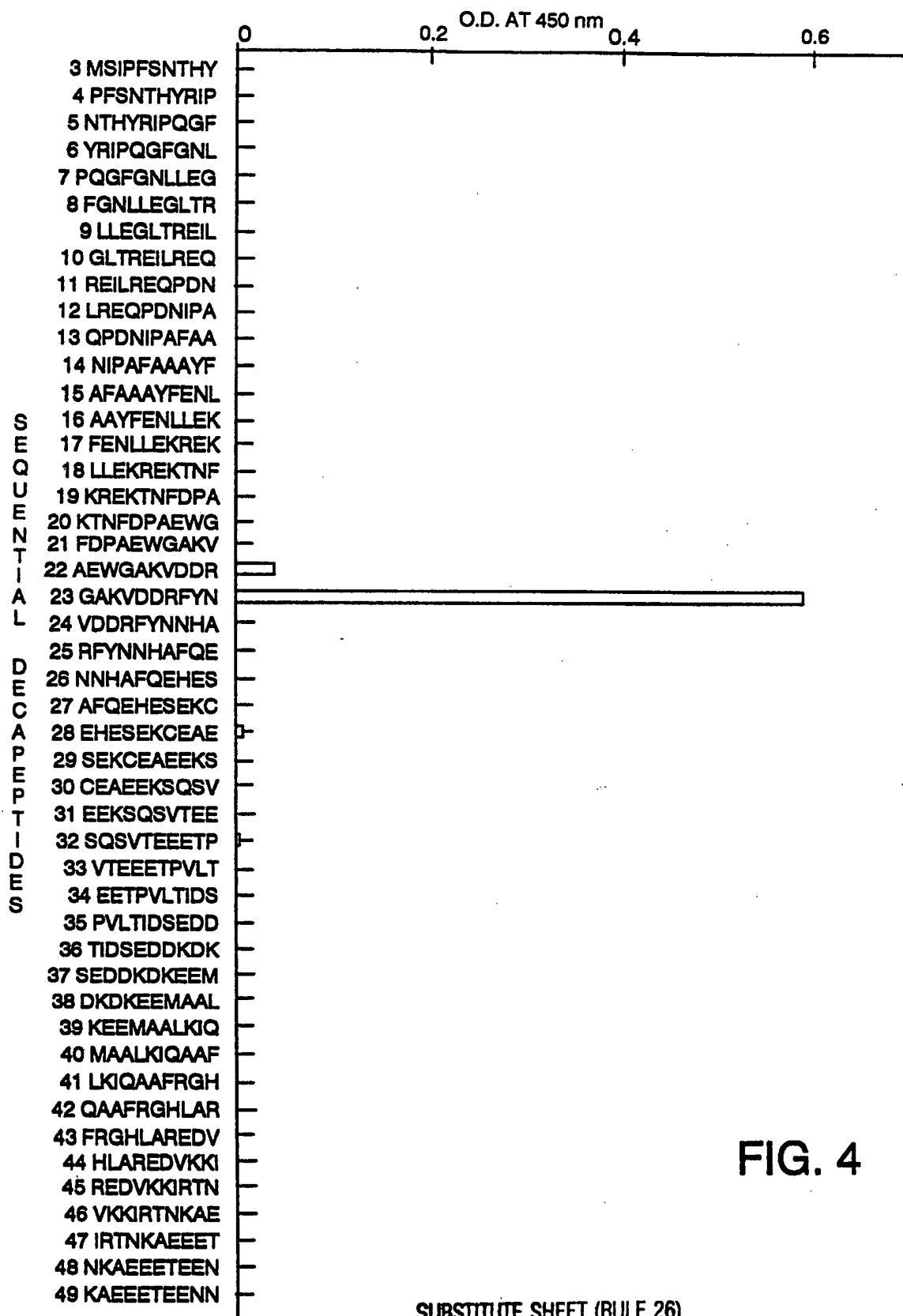


FIG. 3

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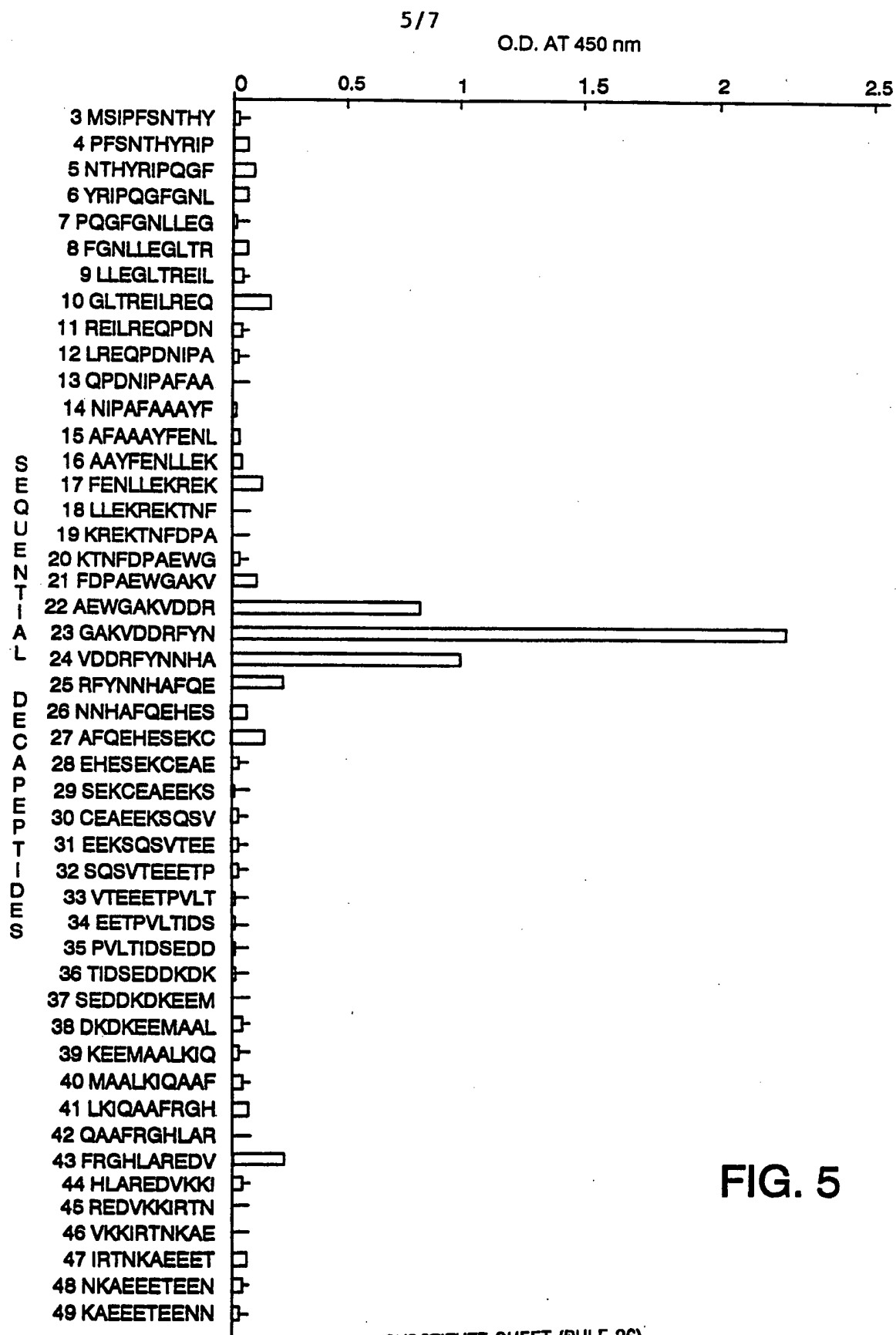
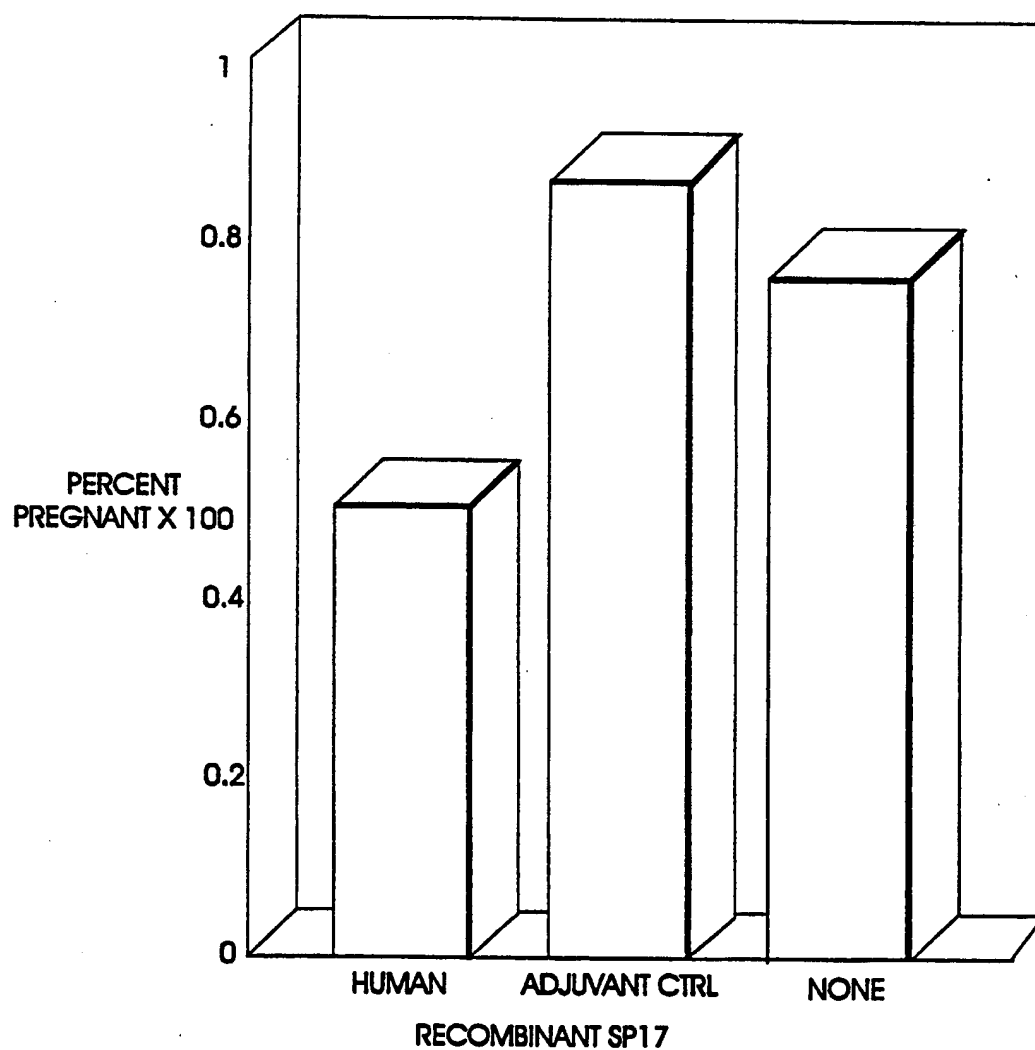


FIG. 5

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FIG. 6



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FIG. 7

| | | | | | |
|---------|-------------------------------|----------------------------------|-------------------|-------|-------------|
| RABSP17 | MSIPFSNTHYRIPQGGNLLGLEGLTREIL | REQPD | NIPAFAAAYFENILLEK | REKTN | FDPAEWGAKVD |
| MUSSP17 | MSIPFSNTHYRIPQGGNLLGLEGLTREIL | REQPD | NIPAFAAAYFENILLEK | REKTS | FDPAEWGAKVE |
| HUMSP17 | MSIPFSNTHYRIPQGGNLLGLEGLTREIL | REQPD | NIPAFAAAYFESILLEK | REKTN | FDPAEWGSKVE |
| | 4 | 28 | 34 | 49 | 55 61 |
| | 1 | | | | |
| RABSP17 | DRFYNNHAFQEHSEKCEA | -EEKSQSVT-EEETPVLTI - DSEDDKDKEE | MAALKQAAFRGH | | |
| MUSSP17 | DRFYNNHAFKEQEQVEKCE | -QELAKSSG-REETPVTPEEESTEEREQEE | AAALKQSLFRGH | | |
| HUMSP17 | DRFYNNHAFEEQEPPEKSD | pQEEESQISGKEETSV-TILDSSEEDKEKEE | VAAVKQAAFRGH | | |
| | 82 | | | 117 | |
| | 137 | | | | |
| RABSP17 | AREDVKKI | RTNKAEEEEENIN- | | | |
| MUSSP17 | AREEVKKM | KSDKNENLKEEADn | | | |
| HUMSP17 | AREEAKKM | KTNSLQNEEKEENK | | | |
| | | 151 | | | |

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US94/13328

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :Please See Extra Sheet.

US CL :Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/69.1, 71.1, 172.3, 252.3; 424/93.2, 184.1, 192.1, 200.1, 811; 536/23.1, 23.5, 25.3

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

INTELLIGENETICS, APS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|-----------|--|-----------------------|
| Y | US, A, 5,175,148 (O'RAND ET AL.) 29 December 1992, see entire document. | 1-28 |
| Y | ANNALS NEW YORK ACADEMY OF SCIENCES, Volume 541, ISSUED 1988, Shabanowitz et al., "Molecular Changes in the Human Zona Pellucida Associated with Fertilization and Human Sperm-Zona Interactions", pages 229-246, see entire document. | 1-28 |

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

| Special categories of cited documents: | |
|--|--|
| *A* | document defining the general state of the art which is not considered to be of particular relevance |
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| *L* | document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) |
| *O* | document referring to an oral disclosure, use, exhibition or other means |
| *P* | document published prior to the international filing date but later than the priority date claimed |
| *T* | later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention |
| *X* | document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone |
| *Y* | document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art |
| *Z* | document member of the same patent family |

Date of the actual completion of the international search

28 FEBRUARY 1995

Date of mailing of the international search report

13 MAR 1995

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/13328

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (6):

A61K 35/48, 35/52, 35/54, 39/00, 39/395; G01N 33/53, 33/68; C07K 14/00, 16/00, 16/18, 16/28, 16/42

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

435/69.1, 71.1, 172.3, 252.3; 424/93.2, 184.1, 192.1, 200.1, 811; 536/23.1, 23.5, 25.3